Diagnostic BioSystems

IHC Made Affordable

Mouse/Rabbit UnoVue Plus™ AP/PermaRed Detection System

Catalog No: UVP-25AR, UVP-100AR, UVP-1000AR
Document #: DS-6023-C
Effective Date: 12/18/2023

Intended Use: For In Vitro Diagnostic Use

The Mouse/Rabbit UnoVue Plus AP/PermaRed Detection System is suitable for use with mouse rabbit IgG and IgM antibodies, both monoclonal and polyclonal. The reagents can be used for manual staining or with automated staining platforms.

Principles of the Procedure: The Mouse/Rabbit UnoVue Plus AP PermaRed Detection System is a non-biotin, one-step Mouse/Rabbit polymer detection system suitable for demonstrating antigens in formalin-fixed paraffin-embedded tissues and cryostat sections. The UnoVue Plus Detection System may also be used with blood smears, cytosmears, and cell preparations. The UnoVue Plus detection kits have been developed by directly labeling anti-mouse and anti-rabbit immunoglobulin with enzymes using a proprietary tandem hyperlabelling technology. This ensures consistent and reproducible immunodetection of mouse and rabbit antibodies with a single reagent. Nuclear, cytoplasmic and membrane antigens in different types of tissues can be detected readily. The single step UnoVue Plus Detection System enables faster staining procedures than traditional two-step methods using biotin and avidin/streptavidin conjugates, with significantly lower background.

Kit Contents Reagents provided are respective number of tests.

Description	Cat # UVP-25AR 25 Tests	Cat # UVP-100AR 100 Tests	Cat# UVP-1000AR 1000 Tests
UnoVue Plus Anti-Mouse/Rabbit AP Polymer	2.5 mL	10 mL	100 mL
PermaRed AutoPlus Buffer	2.5 mL	10 mL	100 mL
PermaRed AutoPlus Chromogen	2.5 mL	10 mL	100 mL

Storage and Handling Store at 2°-8°C away from light. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Stability 12-24 months (see expiration date on reagent bottles).

Composition All reagent components are formulated without azide or thimerosol preservatives. The reagents are provided in ready-to-use format with the exception of PermaRed Buffer and Chromogen.

Material Required But Not Provided Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at www.dbiosys.com.

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Preparation of PermaRed/AP Substrate Working Solution

For Manual Use:

1. Mix equal volumes of buffer and chromogen prior to use on slide.
2. The substrate working solution should be used *within 20 minutes* of preparation. Discard any solution not used after this period.

For Automation Use:

Contact DBS technical support for instrument-specific protocols

Dispose of unused PermaRed/AP Substrate working solution in appropriate waste.
Stream according to local, state or federal regulations.

Precautions

This product is a single-use, non-sterile, in vitro diagnostic device.
i)Wear appropriate personal protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
ii) Interpretation of the results is the sole responsibility of the user.

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

Recommended Staining Protocol

1. Paraffin embedded tissue sections must be deparaffinized with xylene or dewaxing agent and rehydrated with a graded series of ethanol and water washes before staining. Follow the standard dewaxing and rehydration protocol used in your lab.
2. The investigator needs to optimize the dilution and incubation times for primary antibodies.
3. Each immunostaining run should include known positive and negative controls to assure proper functioning of the staining system and aid in valid interpretation of the results.

Typical controls:

Positive Control: A tissue known to contain the desired antigen, which has yielded positive staining in the past.

Negative Controls:

Reagent Controls

A. Substitute normal non-immune serum from the same host animal as the primary antibody (e.g. if using mouse monoclonal primary antibodies, use mouse non-immune serum).
B. Substitute matching host species isotype control for primary antibody
C. Use antigen-adsorbed primary antibody (i.e. antibody reagent which has been adsorbed with the target antigen to remove specific antibody)

Tissue control – A tissue known to *not* contain the desired antigen.

4. Consult the primary antibody supplier for recommended for antigen recovery treatments. Perform epitope recovery pretreatments before starting the staining procedure.
5. Once the slide treatment has been started, DO NOT let tissues or specimens dry. This can cause undesirable background or artifacts.

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STEP	STAINING PROCEDURE:	INCUBATION TIME
1. Pre-Blocking (optional)	A. Add 2 drops (100 µL) or enough volume of Pre-Blocking Solution to cover the tissue section. B. Drain or blot off solution. **Do not rinse**.	**10 min.**
2. Primary Rabbit Antibody	A. Incubate with Primary Antibody, prepared according to the manufacturer's recommended protocol at the desired concentration. Concentrated Primary Antibodies may be diluted using Primary Antibody Diluent. B. Wash slides with 3 changes of Immuno Wash Buffer.	**30 – 60 min.** **3 x 1 min**.
3. UnoVue Plus Rabbit AP Polymer	A. Incubate the tissue with UnoVue Plus Mouse/Rabbit AP Polymer reagent. B. Wash slides with 3 changes of Immuno Wash Buffer.	**20 min.** **3 x 1 min.**
4. PermaRed/AP	A. Prepare the PermaRed/AP substrate working solution (see above). Use within 20 min of preparing working solution. B. Incubate tissue with prepared PermaRed/AP substrate solution. Monitor level of staining to determine optimal time of incubation. C. Rinse slides with 3 changes of water.	**5 – 15 min.** **3 x 1 min.**
5. Counterstain	A. Incubate tissue with Counterstain (e.g. Hematoxylin), according to manufacturer's recommendation or standard laboratory protocol. B. Wash slides with water 3 times, followed by 1 time in Immuno Wash Buffer, then 1 time in water.	**~1 min.** **3 x 1 min H_2O** **1 x 1 min Buffer** **1 x 1 min H_2O**
6. Mount Coverslips	Stained tissue sections can be dehydrated in alcohol and cleared in xylene or xylene substitute and permanently mounted.	

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